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Inactivation of Venezuelan Equine Encephalomyelitis Virus by γ-Radiation

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Exposure of Venezuelan equine encephalomyelitis (VEE) virus (at −70 C) to 6×10^6 r γ-radiation (^{60}Co) resulted in loss of lethality for young adult mice and guinea pigs, and loss of capacity to produce plaques or cytopathic effects in tissue culture. The suckling mouse was more sensitive for detecting live virus in radiated suspensions than was the adult mouse or guinea pig. Live virus was demonstrable in preparations exposed to 6×10^6 r but not in suspensions exposed to 8×10^6 r and more. The rate of inactivation of VEE virus by γ-radiation was an exponential function of the dosage.

It has been reported that γ-rays inactivate the viruses of poliomyelitis, St. Louis encephalitis, western equine encephalitis (1), vaccinia (1, 2), influenza, and mumps (6). The results presented suggest that this method of inactivation destroys the capacity of the virus to produce infectivity in animals, but it does not alter the antigenicity unless large doses of radiation are employed. These studies prompted our investigation on inactivation of Venezuelan equine encephalomyelitis (VEE) virus by exposure to γ-rays.

MATERIALS AND METHODS

Virus. The Trinidad strain of VEE was used for all experiments. Virus was propagated in monolayers of mouse fibroblast strain L cells or in a Maitland-type culture of chick embryo tissue in a chemically defined medium (4). Supernatant fluids were harvested and clarified by centrifugation; samples were stored in sealed ampules or rubber-stoppered bottles at −70 C.

Radiation. Virus suspensions were irradiated at the National Bureau of Standards, by using a 50,000-curie cobalt-60 source emitting radiation at a rate of approximately 7.5×10^6 r/hr. Radiation dosages were calculated from the source intensity and geometry in respect to position of the material being irradiated. Suspensions were irradiated in glass containers sealed in tin cans in a Dewar flask and inserted into a carrier (Fig. 1). The samples were kept frozen with dry ice during exposure to minimize the indirect lethal action of free radicals (8). After exposure, samples were stored at −70 C until tested for presence of active virus. Control samples of virus were exposed to identical conditions but were not irradiated.

Titration of virus suspension. Survival curves of irradiated virus were determined by intracerebral (ic) inoculation of 0.03 ml in 10- to 14-g Swiss mice, and by plaque assay and capacity to induce cytopathic effects (CPE) in mouse fibroblast strain L cells. Samples of radiated virus that were nonlethal for adult mice were inoculated into 250- to 350-g guinea pigs. Each of 15 guinea pigs was administered 0.25 ml of undiluted or a 10^{-1}, 10^{-2}, 10^{-3}, or 10^{-4} dilution of virus. No deaths occurred.

On the basis of data obtained, guinea pigs (250 to 350 g) were inoculated with selected samples of radiated VEE. These samples ranged from those exposed to doses of radiation that failed to inactivate all viable virus, to those that were shown to be nonlethal for adult mice and guinea pigs. The animals received either one or two inoculations given at an interval of 1 week; dosages are given in Table 3. Five animals were bled 3 weeks postinoculation by intracardial puncture, and the level of serum-neutralizing and hemagglutination-inhibiting antibodies was determined. The resistance of the guinea pigs to challenge with virulent homologous virus was determined. A dose of 2.5×10^6 mouse ic lethal doses 50% (MICLD$_{50}$) was administered to each animal. Mice were utilized in similar tests but no serological assays were performed.

Virus suspensions were titrated in suckling mice by inoculation of 0.03 ml via the ic and 0.03 ml via the intraperitoneal (ip) route in the same animal. The brains of mice that died 24 hr or more after inoculation were harvested, ground in a mortar, and suspended in Beef Heart Infusion (Difco). The presence of VEE virus was confirmed by neutralization with VEE antiserum in adult mice.

Serum neutralization test. The serum neutralization (SN) test reported by Smith et al. (8) was used with male mice only.

Hemagglutination inhibition test. Guinea pig sera were tested by the microtiter technique using a VEE hemagglutinin antigen inactivated with β-propiolactone.

RESULTS

Figure 2 shows the effect of various doses of γ-radiation on the survival of VEE virus as de-

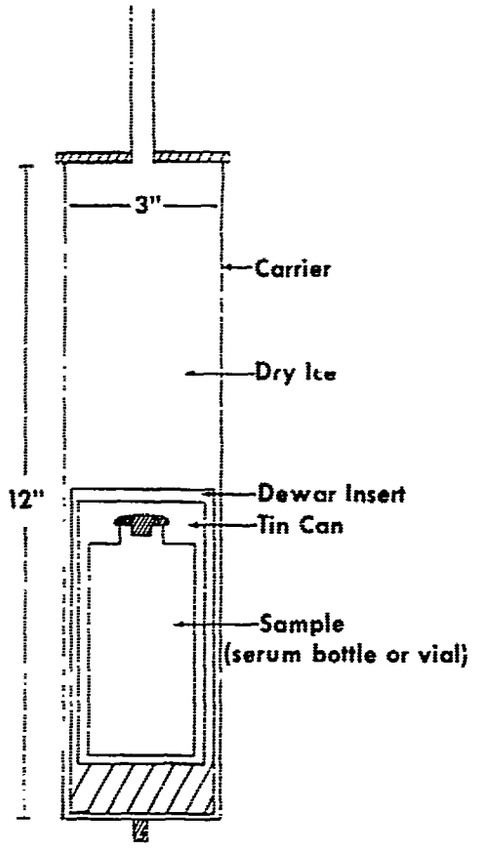

FIG. 1. *Container arrangement for irradiating samples.*

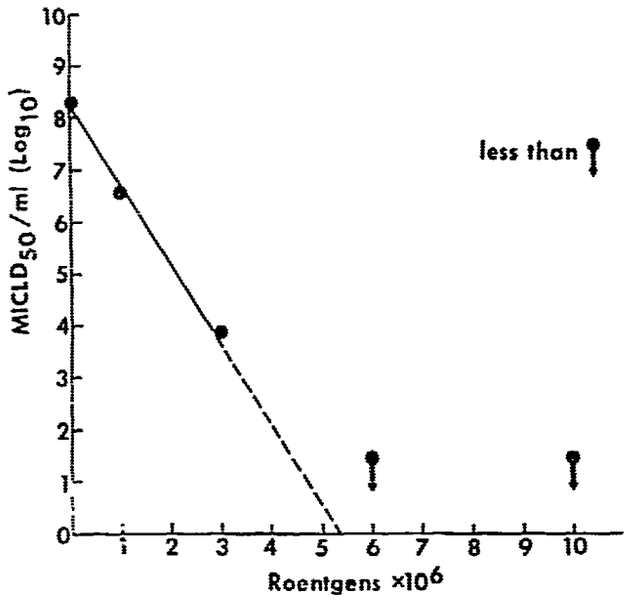

FIG. 2. *Survival curve of Venezuelan equine encephalomyelitis virus obtained by irradiation with γ rays.*

termined by assay in adult mice. The median lethal dose (LD_{50}) values, calculated according to the Reed and Muench formula (7), are plotted on a logarithmic scale as a function of radiation dose. The results show that the rate of inactivation is expressed as an approximately straight line, indicating that the surviving fraction is an exponential function of the dose. Deaths did not occur when young adult mice were inoculated with virus that had been exposed to radiation doses of 6×10^6 r or greater (Table 1). Similar results were obtained with guinea pigs (Table 2). The capacity to produce plaques or CPE was lost in suspensions exposed to 6×10^6 r or greater.

The results of the assays in suckling mice and of the hemagglutination-inhibition (HAI) and SN tests from a typical experiment are shown in Table 2. The test with suckling mice indicated the presence of live virus in the suspensions irradiated with 5×10^6 and 6×10^6 r, but not in those suspensions exposed to larger doses. The virus exposed to 5×10^6 r was lethal for 20% of guinea pigs but not for adult mice. High SN indices were obtained in all guinea pigs which were administered two inoculations of virus that had been exposed to radiations ranging from 5×10^6 to 10×10^6 r. A significantly lower SN antibody response was obtained in guinea pigs inoculated with virus exposed to 16×10^6 r. Assuming that virulence for suckling mice is correlated with the amount of live virus present in the irradiated suspensions, the lowest HAI antibody responses were obtained with the preparation containing the greatest amount of live virus. The protective potency determined by antigen extinction assay is expressed here as the effective dose (ED_{50}), the volume of undiluted irradiated virus suspension inoculated per dose that protected 50% of the test animals. The ED_{50} for mice was the same for preparations exposed to radiation doses ranging from 5×10^6 to 8×10^6 r, but was greater at higher radiation levels.

Table 3 lists the SN antibody response and the ED_{50} in milliliters obtained in guinea pigs that received either one or two inoculations of radiated virus. The primary antibody response was at a low level in the animals that received only one inoculation. The second inoculation caused an increase in antibody in 50% of the animals. It

TABLE 1. *Inactivation of Venezuelan equine encephalomyelitis virus by γ-irradiation as measured by plaque formation, cytopathic effects, and mouse lethality*[a]

Radiation dose × 10^5 r	$MICLD_{50}$[b] (log base 10)	Plaque-forming units[c] (log base 10)	Cytopathic effects[c] (log base 10)
0	10.0	8.3	8.3
1	7.6	6.0	7.5
3	4.1	2.8	3.5
6	<1.5	<1.0	<1.0
10	<1.5	<1.0	<1.0

[a] Experiment HV-1, L-cell culture.
[b] The mouse intracerebral lethal dose, 50% ($MICLD_{50}$) per ml was 10.0 prior to radiation.
[c] Performed by H. J. Hearn, Jr.

TABLE 2. *Antigenicity and infectivity of irradiated Venezuelan equine encephalomyelitis virus*[a]

Radiation dose × 10^6 r	Suckling mice		Adult mice 10 to 14 g		Guinea pigs	Guinea pig serum[b]	
	Dead/total	Live virus	IPLD$_{50}$/ml (log base 10)	ED$_{50}$[c] (ml)	IPLD$_{50}$/ml (log base 10)	HAI[d]	SN index[e]
5	21/25	+	<0.6	0.003	<0.6[f]	121[g]	>5 × 10^6
6	3/25	+	<0.6	0.003	<0.6	643	>5 × 10^6
8	0/25	−	<0.6	0.003	<0.6	845	>5 × 10^6
10	0/25	−	<0.6	0.04	<0.6	368	8 × 10^3 to >5 × 10^6
16	0/25	−	<0.6	0.04	<0.6	453	1.6 × 10^2 to 5 × 10^3

[a] Experiment MR 27. Maitland-type chick embryo culture. Mouse intracerebral lethal dose, 50% per ml was 9.9. Mouse intraperitoneal lethal dose, 50% per ml was 9.6 prior to radiation.
[b] Received 0.75 ml total inoculum in two inoculations.
[c] Based on 0.5 ml total inoculum in two inoculations.
[d] HAI = hemagglutination-inhibition.
[e] SN = serum neutralization.
[f] Three of 15 died in group that received undiluted treated virus.
[g] Geometric mean of HAI titers. Reciprocal of dilution.

should be noted that Tables 2 and 3 list the results obtained by inoculation of two different virus preparations, and that the guinea pigs listed in Table 2 received a total of 0.25 ml more virus than those listed in Table 3.

DISCUSSION

The results of tests for inactivation of VEE virus measuring the capacity of the virus to produce CPE, plaques, or death of adult mice and guinea pigs, suggest that active virus was not present in suspensions exposed to 6 × 10^6 r of γ-radiation. However, live virus was detected in this preparation by inoculating suckling mice by both the ic and the ip routes. Suckling mice have been reported to be considerably more sensitive to infection with VEE virus than either hamster kidney or monkey kidney tissue cells (3). It is apparent from our experiments that the suckling mouse is more sensitive to the lethal action of irradiated VEE virus than either the adult mouse or the guinea pig.

The high levels of SN and HAI antibodies obtained in the guinea pig listed in Table 2 might be due either to an inapparent infection with live virus or to the stimulation of antibody production upon introduction of additional inactivated virus in the second inoculation. The latter would appear to be the more logical explanation. The data listed in Table 3 show that one inoculation was not sufficient to induce significant antibody titers; a second inoculation resulted in a sharp rise in the level of neutralizing antibody. These results also support the conclusion that live virus was not present in sufficient quantity to produce infection, because it would be expected that significant levels of antibody would have resulted from a single inoculation.

The reduced antibody response that occurred at radiation doses of 10 × 10^6 r or higher may be attributable to breakdown of antigenic material; this phenomenon has been reported to occur with the viruses of influenza (3) and vaccinia (5).

TABLE 3. *Antibody response of guinea pigs to inoculation with irradiated Venezuelan equine encephalomyelitis virus*[a]

Radiation dose × 10^6 r	Effective dose, 50% (ml)		Serum neutralization index	
	One inoculation (0.5 ml)	Two inoculations (0.25 ml × 2)	One inoculation (0.5 ml)	Two inoculations (0.25 ml × 2)
6	0.166	0.008	20	20
			39	10,000
			390	16,000
			0	>63,000
			0	10
8	0.33	0.008	10	>63,000
			16	20
			10	2,000
			10	20
			—	10
10	>0.5	0.008	2	100
			0	15
			2	31
			0	—
			3	—

[a] Experiment MR 25. Maitland-type chick embryo culture. Mouse intracerebral lethal dose, 50% (MICLD$_{50}$) per ml was 9.0 prior to radiation.

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